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Cytology and breeding behavior of interspecific hybrids and induced amphiploids of *Zinnia elegans* and *Zinnia angustifolia*[1]

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Cytological studies were performed on interspecific hybrids and induced amphiploids of *Zinnia angustifolia* HBK ($2n = 22$) and *Zinnia elegans* Jacq. ($2n = 24$) to ascertain their potential in serving as intermediaries in the transfer of genes for disease resistance. Partial fertility was restored in sterile F_1 hybrids ($2n = 23$) through colchicine treatment of axillary buds. Lagging univalents and irregular distribution of chromosomes to the gametes were the major contributing factors to the sterility observed among the F_1 hybrids. Bivalent associations in the F_1 indicated partial homology between parental genomes. The induced amphiploids ($2n = 46$) formed predominantly bivalents at metaphase I owing to the suppression of pairing between homoeologous chromosomes. Consequently, these segmental allopolyploids resembled diploids in their cytological and genetic behavior and bred true to their intermediate condition with little or no segregation in later generations. It is postulated that the gene(s) controlling chromosome pairing is derived from *Z. elegans*. The cytological and genetic performance of colchicine-induced amphiploids of *Z. elegans* and *Z. angustifolia* suggest considerable potential for the improvement of *Z. elegans* cultivars with respect to disease resistance and the immediate stabilization of characters through genetic uniformity.

TERRY-LEWANDOWSKI, V. M., G. R. BAUCHAN et D. P. STIMART. 1984. Cytology and breeding behavior of interspecific hybrids and induced amphiploids of *Zinnia elegans* and *Zinnia angustifolia*. Can. J. Genet. Cytol. **26**: 40–45.

Des études cytologiques ont été poursuivies sur des hybrides interspécifiques et sur des amphiploïdes induites de *Zinnia angustifolia* HBK ($2n = 22$) et de *Zinnia elegans* Jacq. ($2n = 24$) pour vérifier leur potentiel comme intermédiaires pour le transfert de gènes de résistance aux maladies. Une fertilité partielle a été rétablie chez des hybrides F_1 stériles ($2n = 23$) par traitement à la colchicine des bourgeons axillaires. Des univalents traînards et une distribution irrégulière des chromosomes vers les gamètes ont été les principaux facteurs de stérilité chez les hybrides F_1. Des associations de bivalents chez les F_1 ont indiqué l'existence d'une homologie partielle entre les génomes des parents. Les amphiploïdes induits ($2n = 46$) ont formé, de façon prédominante, des bivalents à la métaphase I du fait de la suppression d'appariement entre les chromosomes homologues. Conséquemment, ces allopolyploïdes segmentés ressemblaient à des diploïdes dans leur comportement cytologique et génétique, et cet état intermédiaire permettait une fécondation vraie, avec peu ou pas de ségrégation chez les générations ultérieures. Il est postulé que le ou les gènes qui contrôlent l'appariement des chromosomes sont dérivés de *Z. elegans*. La performance cytologique et génétique des amphiploïdes induites à la colchicine de *Z. elegans* et de *Z. augustifolia* suggère qu'il existe un potentiel considérable pour l'amélioration des cultivars de *Z. elegans* en fonction de la résistance aux maladies et de la statilisation immédiate des caractères par suite de l'uniformité génétique.

[Traduit par le journal]

Introduction

Zinnia elegans Jacq. is a popular garden ornamental plant throughout its worldwide range of cultivation. However, its susceptibility to *Erysiphe cichoracearum* DC. ex Merat (causal organism of powdery mildew), *Alternaria zinniae* Pape (causal organism of alternaria blight), and *Xanthomonas campestris* pv. *zinniae* Hopkins & Dowson (causal organism of bacterial leaf and flower spot) has recently posed an economic threat to commercial seed producers (L. Drewlow, personal communication)[4]. One source of potentially valuable genes for resistance to the three major pathogens of *Z. elegans* and subsequent improvement of cultivars is *Zinnia angustifolia* HBK (syn. *Z. linearis* Benth.) (Torres 1963; Lipschutz 1965; Strider 1976).

Successful hybridizations between *Z. elegans* ($2n = 24$) (Torres 1963; Ramalingam et al. 1971; Gupta and Koak 1976) and *Z. angustifolia* ($2n = 22$) (Olorode 1970; Ramalingam et al. 1971) have been reported (Ramalingam et al. 1971; Boyle and Stimart 1982), although partial to complete sterility in the F_1 placed serious limitations on subsequent use. Boyle and Stimart (1982) circumvented the sterility barrier by col-

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chicine treatment of selected F_1 hybrids. Phytopathological studies performed in this laboratory demonstrated that the induced amphiploids possess high levels of resistance to *E. cichoracearum*, *A. zinniae*, and *X. campestris* pv. *zinniae* (Terry-Lewandowski and Stimart 1983). In addition, all advanced generations of amphiploids failed to segregate for resistance to *E. cichoracearum*; there was a very high degree of genetic uniformity for this trait. It was suggested that backcrossing the amphiploids to *Z. elegans* should result in the transfer of multiple genes for disease resistance from *Z. angustifolia* to commercially acceptable cultivars of *Z. elegans*. The objectives of this investigation were to ascertain the potential of this germplasm by examining the cytology, fertility, and breeding behavior of interspecific hybrids and fertile derivatives of *Z. elegans* and *Z. angustifolia*.

Materials and methods

Two interspecific hybrids, one from each reciprocal cross, were selected for study from stock plants maintained at the University of Maryland, MD (Boyle and Stimart 1982). Fertility was restored in sterile F_1 hybrids of *Z. elegans* 'Whirligig' × *Z. angustifolia* (F_1-W) and *Z. angustifolia* × *Z. elegans* 'Cherry Ruffles' (F_1-CR) by treating axillary buds daily with droplets of 0.1% aqueous colchicine for 5 days following shoot tip removal. Polyploid sectors, which were recognized by plump, dehiscent anthers, stainable pollen, and seed set, were designated as the C_0 generation. Generations were advanced from the C_0 through the C_2 by controlled self-pollination of inflorescences maintained under a fabric tent (24% daylight reduction). Members of the C_2 generation, designated as C_2-W and C_2-CR, respectively, were used for cytological examination. Somatic chromsome counts were made from excised root tips pretreated for 4 h in 0.1% aqueous colchicine and fixed for 24 h in Carnoy's fluid (6:3:1 of 95% ethanol, chloroform, and glacial acetic acid, respectively). Root-tip squashes were prepared by hydrolyzing the meristematic tissue in 1 *N* HCl at 60°C for 15 min, staining in Feulgen for 1 h, and mounting in 1% acetocarmine.

Immature flower buds for cytological analyses were fixed in Carnoy's fluid (6:3:1) and stored at 4°C. Pollen mother cells from disk florets of approximately 1.2 to 1.4 mm in length were stained in acetocarmine. All cytological data were collected from semipermanent slides.

Pollen viability of F_1-CR, F_1-W, C_2-CR, and C_2-W was estimated by counting the dark blue spherical pollen grains from a composite sample of 2000 pollen grains per plant type mounted in aniline blue – lactophenol.

Segregation for morphological traits was investigated in the greenhouse to further substantiate the observed cytological behavior. Twelve C_2-W families and eight C_2-CR families were arranged in a completely randomized experimental design with 24 replications per family. An analysis of variance was performed on days to flower, flower diameter, number of rays, ray length and width, and leaf length and width. Significance of *F* values would indicate genotypic differences among C_2 families.

Results

Examination of root-tip cells of *Z. angustifolia* and *Z. elegans* 'Cherry Ruffles' and 'Whirligig' revealed respective somatic chromosome numbers of $2n = 22$ and $2n = 24$. The meiotic behavior of the parental species was normal, *Z. elegans* forming 12 and *Z. angustifolia* forming 11 bivalents at metaphase I (MI). Chromosome separation in subsequent stages of meiosis was also very regular.

The parental species exhibited pronounced differences in chromosome size. *Zinnia elegans* had chromosomes which ranged from 2.7 to 3.5 μm in length, whereas, *Z. angustifolia* had chromosomes which ranged from 1.4 to 2.0 μm in length. This size differential was readily detected in both somatic and pollen mother cells of F_1 hybrids and amphiploids (Figs. 1 and 5).

Somatic chromosome counts of the F_1 hybrids and amphiploids confirmed our expectations of chromosome number and ploidy level. The F_1 hybrids possessed 23 chromosomes (Fig. 1) which were doubled to 46 in the colchicine-induced amphiploids (Fig. 5). Unlike the parental species, F_1-CR and F_1-W exhibited considerable meiotic irregularities. The most frequent observation was 23 univalents at MI, although a variable number of bivalents ranging from one to six were observed (Fig. 2; Table 1). The high frequency of univalents resulted in irregular separation of chromosomes at anaphase I (AI) (Fig. 3). The presence of laggards further disrupted later stages of meiosis, with multiple poles forming at telophase II (TII) (Fig. 4) and micronuclei distributed among the quartets.

Stabilization of chromosome pairing occurred upon doubling the somatic complement of the F_1 hybrids. Both C_2-CR and C_2-W regularly formed 23 bivalents at MI (Fig. 6); however, a low percentage of univalents was also observed at this stage (Tables 2 and 3). No trivalent or quadrivalent associations were observed. Subsequent to MI, meiosis in C_2-CR (Table 3) was somewhat more irregular in terms of laggards (Fig. 7) and micronuclei (Fig. 8) than it was in C_2-W (Table 2). Normal separation of chromosomes in the first division occurred in 69% of the cells of C_2-W and in 30% of the cells of C_2-CR. One or two micronuclei were observed in 13% of the quartets of C_2-W, in contrast with one to six micronuclei per quartet in 89% of the quartets of C_2-CR (Tables 2 and 3).

Pollen stainability of F_1-CR and F_1-W was less than 1%, indicating complete sterility. Colchicine treatment restored partial fertility in the amphiploids with pollen viability ranging from 42% in C_2-W to 37% in C_2-CR.

An analysis of variance performed on C_2-CR and C_2-W families demonstrated a very high degree of genetic uniformity for days to flower, flower diameter, number of rays, ray length and width, and leaf length

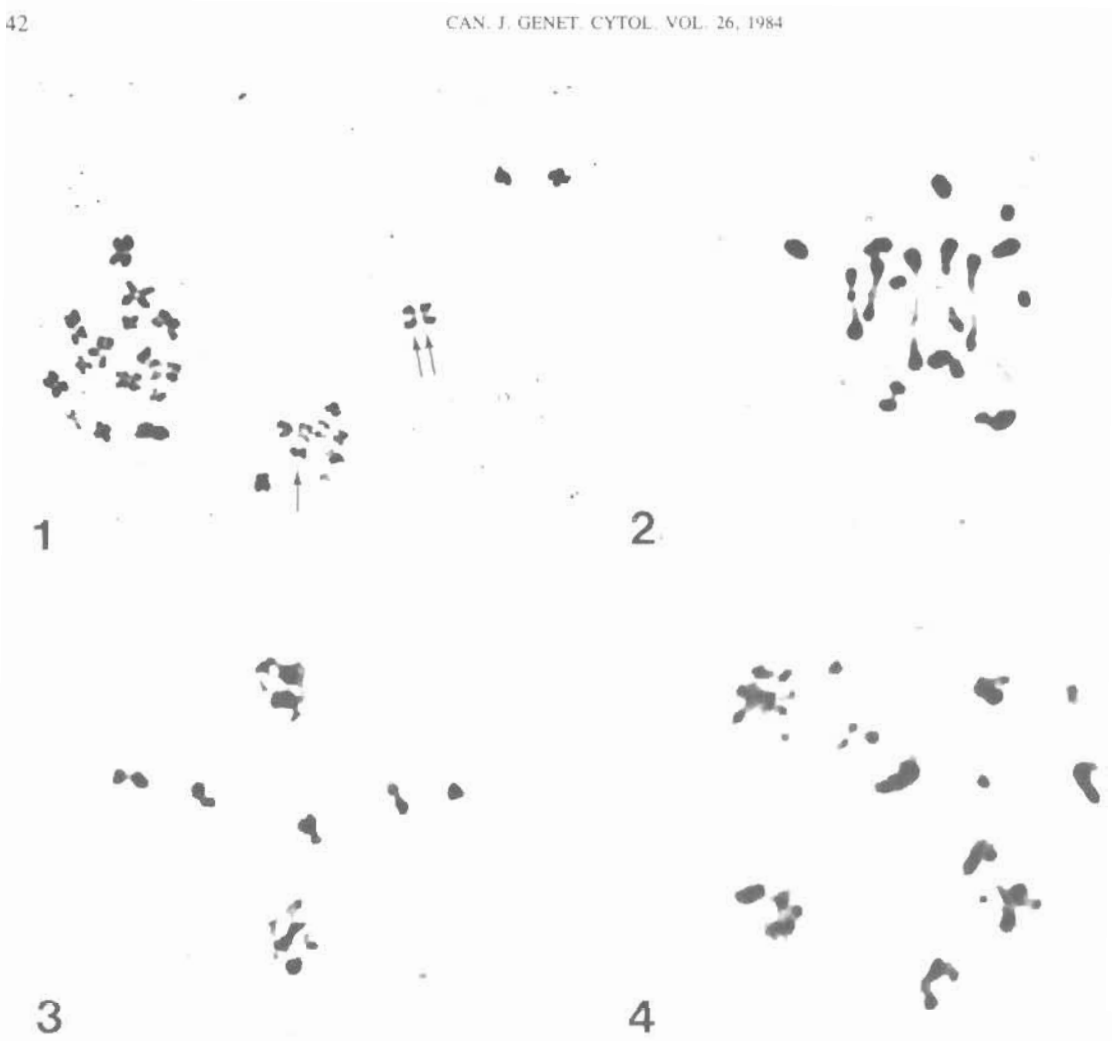

FIG. 1. Somatic chromosomes of F_1-CR ($2n = 23$). Note the chromosome size difference of *Z. angustifolia* (arrow) and *Z. elegans* (double arrows). ×1575. FIG. 2. Metaphase I in F_1-CR with 4 bivalents and 15 univalents. ×1375. FIG. 3. Anaphase I in F_1-CR with five laggards on the metaphase plate. ×1575. FIG. 4. Telophase II in F_1-W showing multiple poles. ×1975.

and width. The lack of significance for the majority of the quantitative traits measured illustrates the absence of segregation among C_2 families (Table 4).

Discussion

Chromosome disturbances during meiosis of F_1 hybrids (F_1-CR and F_1-W) and induced amphiploids (C_2-CR and C_2-W) were manifest in reduced pollen viability. Lagging univalents leading to the formation of gametes with highly unbalanced chromosome complements were the major contributing factors to sterility in the F_1 hybrids. Although colchicine treatment frequently results in a high degree of fertility in derived amphiploids, the C_2 amphiploids examined in this study were only partially fertile. This signficant reduction in fertility is presumably the result of precocious disjunction of bivalents with the consequent formation and irregular distribution of univalents.

Based on the cytological behavior of induced autotetraploids of *Z. angustifolia* ($2n = 44$), Menon et al. (1969) suggested that the diploid species ($2n = 22$) was composed of two basic sets of five and six chromosomes. These basic numbers presumably originated from the hybridization of two ancestral species ($2n = 10$ and $2n = 12$). In agreement with Ramalingam et al. (1971), our observations of one to six bivalents at MI in F_1 hybrids of *Z. angustifolia* and *Z. elegans* indicate partial homology between genomes. Ramalingam and his co-workers proposed that the set of six chromosomes in the gametic complement of *Z. angustifolia* are partially homologous to a similar set of six chromosomes in *Z. elegans*, thus supporting the

TABLE 1. Chromosome associations at metaphase I of F_1 hybrids of *Z. elegans* and *Z. angustifolia*

F_1 hybrid	Chromosome associations I	Chromosome associations II	No. of cells	%
Z. angustifolia × *Z. elegans* 'Cherry Ruffles' (F_1-CR)	23	0	37	31
	21	1	23	19
	19	2	27	23
	17	3	19	16
	15	4	8	7
	13	5	2	2
	11	6	2	2
Total			118	100
Z. elegans 'Whirligig' × *Z. angustifolia* (F_1-W)	23	0	27	26
	21	1	23	22
	19	2	21	20
	17	3	13	12
	15	4	12	11
	13	5	6	6
	11	6	3	3
Total			105	100

TABLE 2. Chromosome pairing in amphiploid hybrids of *Z. elegans* 'Whirligig' × *Z. angustifolia*

Diakinesis/metaphase I				Anaphase I/telophase I			Quartets		
Chromosome associations I	Chromosome associations II	No. of cells	%	Laggards per cell	No. of cells	%	Micronuclei per cell	No. of cells	%
0	23	108	90	0	115	69	0	137	87
2	22	11	9	1	34	20	1	15	10
4	21	1	1	2	16	9	2	5	3
				3	1	1			
				6	1	1			
Total		120	100		167	100		157	100

TABLE 3. Chromosome pairing in amphiploid hybrids of *Z. angustifolia* × *Z. elegans* 'Cherry Ruffles'

Diakinesis/metaphase I				Anaphase I/telophase I			Quartets		
Chromosome associations I	Chromosome associations II	No. of cells	%	Laggards per cell	No. of cells	%	Micronuclei per cell	No. of cells	%
0	23	83	83	0	17	30	0	12	11
2	22	16	16	1	30	53	1	22	20
4	21	1	1	2	6	10	2	65	58
				3	3	5	3	4	3
				5	1	2	4	7	6
							5	1	1
							6	1	1
Total		100	100		57	100		112	100

theory of allosyndetic pairing. Our results, however, do not conform with their reports of an occasional trivalent at MI.

From the data presented in this study, two possible genome combinations are proposed for the parental species. Depending on whether the homoeologus chromosomes are undergoing autosyndetic or allosyndetic pairing in the F_1 hybrid, *Z. elegans* may be a segmental

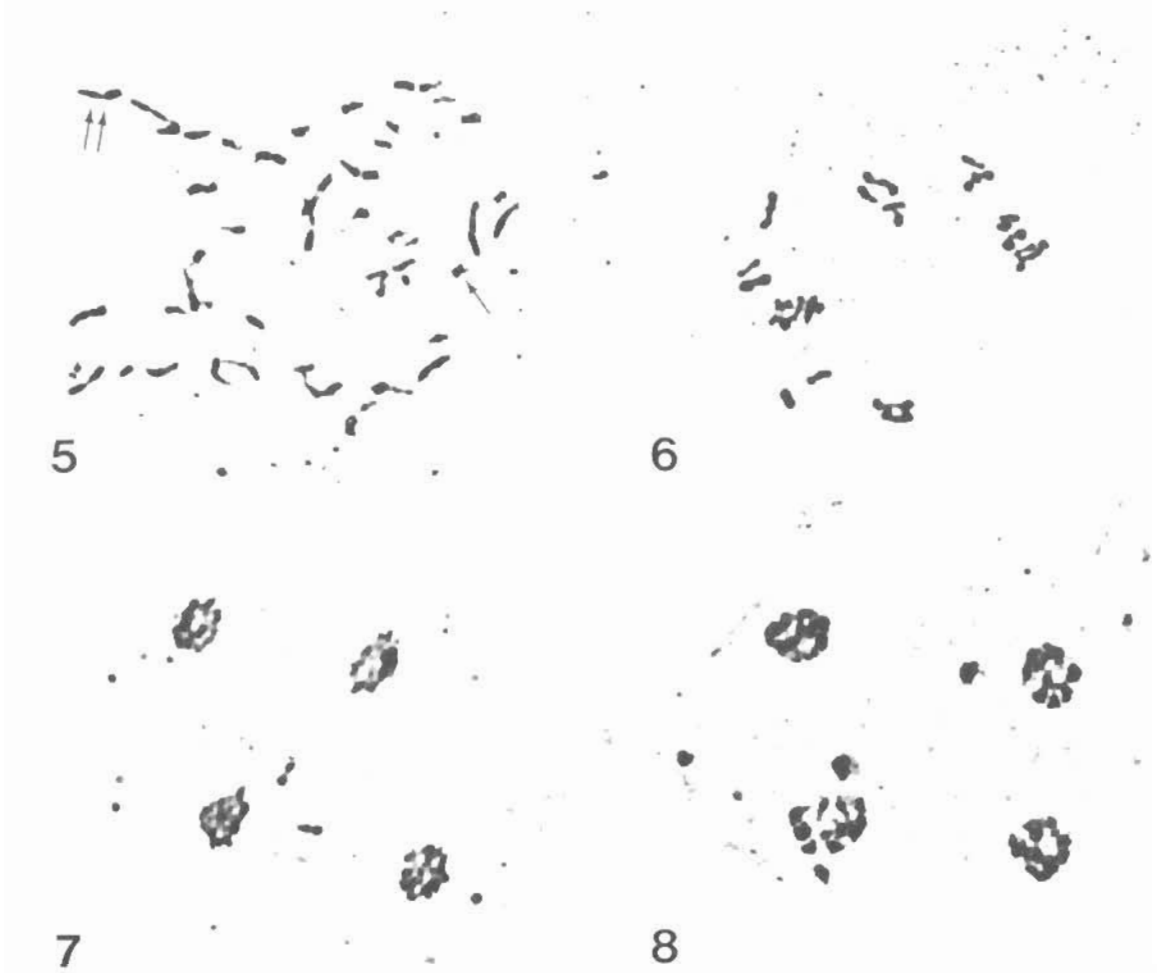

FIG. 5. Somatic chromosomes of the amphiploid C_2-CR ($2n = 46$). Note the chromosome size difference of *Z. angustifolia* (arrow) and *Z. elegans* (double arrows). ×1375. FIG. 6. Metaphase I in C_2-CR with 23 bivalents. ×1000. FIG. 7. Telophase II in C_2-CR with two laggards. ×1200. FIG. 8. Quartet stage in C_2-CR with four micronuclei. ×1200.

TABLE 4. C_2 family segregation for morphological characters of *Z. elegans* and *Z. angustifolia* amphiploids

Character	*F* value	
	C_2-CR[a]	C_2-W[b]
Days to flower	1.69	1.38
Flower diameter	1.37	1.07
No. of rays	4.45[c]	1.23
Ray length	1.96	0.86
Ray width	1.15	0.73
Leaf length	2.25	0.55
Leaf width	1.84	1.14

[a]*Z. angustifolia* × *Z. elegans* 'Cherry Ruffles' (C_2 generation).
[b]*Z. elegans* 'Whirligig' × *Z. angustifolia* (C_2 generation).
[c]Significant ($p < 0.01$).

allotetraploid ($A_eA_eA'_eA'_e$) or a genomic allotetraploid ($A_eA_eB_eB_e$), respectively. *Zinnia angustifolia* is presented as a genomic allotetraploid in both cases. Based on genome homology alone and in the absence of genetic influence on chromosome pairing, genomic formulae and chromosome associations in the F_1 and derived amphiploids are summarized in Table 5. In this system, homoeologous and homologous chromosomes of amphiploids are expected to associate as multivalents at MI.

The absence of multivalent associations in the colchicine-induced amphiploids examined in this study implies genetic control of chromosome pairing (Table 5). This phenomenon has previously been demonstrated in wheat (Riley and Chapman 1958; Feldman 1966) and in barley (Rajhathy et al. 1964; Starks and Tai 1974). When a single dose of the gene or genes

TABLE 5. Possible genome formulae and chromosome pairing with *Z. elegans* as a segmental and a genomic allotetraploid

Parents		F_1 hybrid		Amphiploid		
					Expected maximum pairing	
Z. elegans	*Z. angustifolia*	Formula	Expected maximum pairing	Formula	Without genetic control	With genetic control
$A_eA_eA'_eA'_e$	$C_aC_aD_aD_a$	$A_eA'_eC_aD_a$	6 II + 11 I	$A_eA_eA'_eA'_eC_aC_aD_aD_a$	6 IV + 11 II	23 II
$A_eA_eB_eB_e$	$A'_aA'_aD_aD_a$	$A_eA'_aB_eD_a$	6 II + 11 I	$A_eA_eA'_aA'_aB_eB_eD_aD_a$	6 IV + 11 II	23 II

controlling pairing is present, homoeologous chromosomes will associate to varying degrees at MI. However, when a double dose is present, as in the amphiploids, fully homologous chromosomes will preferentially pair with one another, and completely eliminate homoeologous pairing (Feldman 1966). Consequently, the amphiploids will form exclusively bivalents at meiosis and breed true for intermediate morphological and ecological characteristics (Table 5). According to Stebbins (1950), they may be classified as segmental allopolyploids which resemble diploids with respect to regularity of chromosome pairing during meiosis and constancy of genetic behavior. Little or no segregation is expected in subsequent generations. This observed cytological behavior is further substantiated by the lack of segregation for resistance to *E. cichoracearum* (Terry-Lewandowski and Stimart 1983) and for morphological traits among C_2 families (Table 4).

The gene or genes controlling pairing may have originated from *Z. elegans*. Gupta and Koak (1976) found that colchicine-induced tetraploids of *Z. elegans* exhibited preferential pairing during meiosis and a very low frequency of quadrivalent associations. Whereas, colchicine-induced tetraploids of *Z. angustifolia* form multivalents (Menon et al. 1969).

Ramalingan et al. (1971) produced karotypes of *Z. angustifolia* and *Z. elegans*, which showed that *Z. angustifolia* had chromosomes which were larger than those of *Z. elegans* with some overlap in size between the species. This is contrary to what was observed in this study. The chromosomes of *Z. elegans* were approximately twice the size of *Z. angustifolia* and there did not appear to be any overlap in size. Therefore, based on the present observations, this size differential may provide further evidence for auto- versus allo-syndetic pairing inthe F_1 hybrids.

The cytological and genetic performance of colchicine-induced amphiploids of *Z. elegans* and *Z. angustifolia* suggest considerable potential for the introduction of genes for disease resistance into cultivated forms. The transfer of desirable traits may best be accomplished by backcrossing to *Z. elegans* at the tetraploid level. Intercrossing the amphiploids may serve the dual purpose of expanding the currently narrow gene pool and creating new genotypic combinations that will improve the value and usefulness of this germplasm.

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